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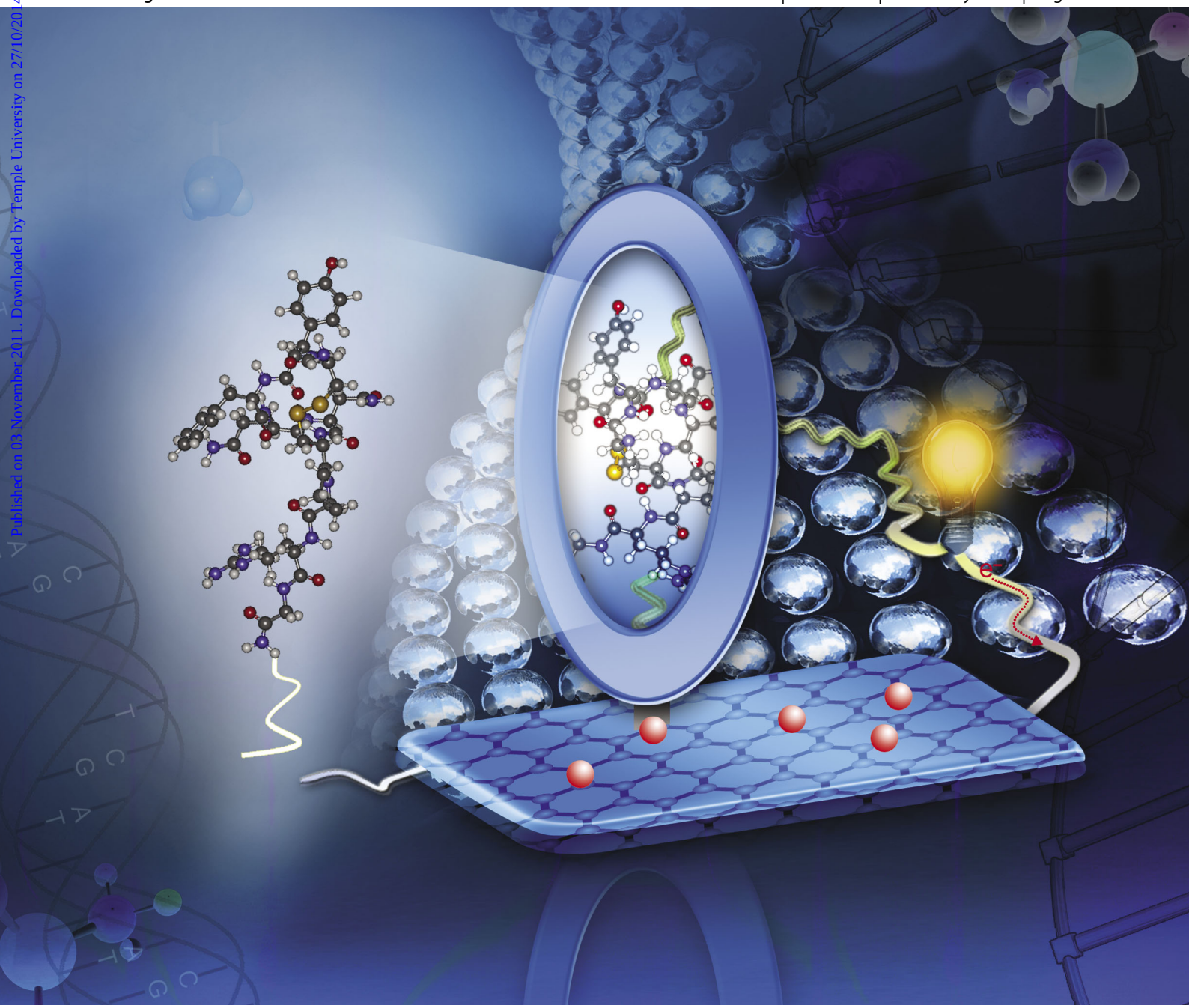

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Target-induced conjunction of split aptamer as new chiral selector for oligopeptide on graphene–mesoporous silica–gold nanoparticle hybrids modified sensing platform†

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A new electrochemical label-free biosensor based on target-induced conjunction of a split aptamer as new chiral selector for oligopeptide using graphene–mesoporous silica–gold NP hybrids (GSGHs) as magnified sensing platform is firstly reported, which showed high sensitivity and selectivity for the detection of D-vasopressin (D-VP).

It is well known that two enantiomers of the same chiral molecule may have totally different physiological behaviors. One of the two enantiomers may be pharmaceutically active, while the other may be inactive or even toxic. The separation and detection of enantiomers are of great importance in pharmaceutical or biological fields. A recent advance revealed that a molecular-engineered DNA aptamer could effectively bind onto the D-enantiomer of an oligopeptide with high affinity and without significant affinity for the L-enantiomer, which provides new opportunity for the effective detection of oligopeptides through the new sensing technique.[1] The oligopeptide, arginine vasopressin (VP), is a neurohypophysial hormone found in most mammals (including humans), which controls the reabsorption of molecules in tubules of the kidneys.[2] VP also plays an important role in homeostasis and the regulation of water, glucose and salts in the blood. Some VP is released directly into the brain where it is important in social behavior and bonding. For a hemorrhagic shock patient, the progression to late phase hemorrhagic shock can be indicated by a marked decrease of plasma VP levels.[3] Replenishing VP can also serve to rapidly increase arterial pressure, thereby stabilizing the patient.[4] The detection of one enantiomer of VP is very important because the two enantiomers possess totally different physiological behaviors.[5] Therefore, developing high-sensitivity analytical strategies for effective detection of D-VP is highly desirable and has been an important research direction. Erickson's group has reported an optofluidic surface enhanced Raman spectroscopy (SERS) device for on-chip detection of vasopressin using an aptamer based binding assay.[6] More recently, Nguyen *et al.* demonstrated label-free microfluidic aptasensors that specifically detect vasopressin by using matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS).[7] However, these sensing devices need special expensive apparatus, and the design of sensor is complicated and time-consuming.

On the other hand, coupling aptamers with graphene and its derivatives has brought a new research direction for the analyst to develop interesting sensing devices for different targets by the use of the novel chemical and physical properties of graphene such as large surface area and excellent conductivity, *etc.*[8] For instance, our group presented the first demonstration of employing graphene as a new substrate for developing high-sensitivity surface plasmon resonance (SPR) electrochemical sensors for thrombin assay.[8b] Yang's group[8c] found that graphene oxide could bind and quench a dye-labeled single-stranded DNA (ssDNA) probe. When ssDNA was combined with target, the fluorescence of the dye could be recovered. Based on this principle, a high-sensitivity GO-based thrombin sensing was established. Our group[8d] further found that hemin–graphene hybrid nanosheets (H-GHs) were interesting materials with intrinsic peroxidase-like activity. By using the unique peroxidase-like properties of H-GHs, a label-free colorimetric detection system for single nucleotide polymorphism was developed, which was mainly based on the different abilities of the H-GHs to differentiate ss-DNA and double-stranded DNA (ds-DNA). All these studies reveal that graphene or its derivatives can act to magnify or label components to improve the detection limit of targets.

Here, we report for the first time a simple and sensitive electrochemical sensor for distinguishing enantiomers of VP by using a designed split aptamer as a new chiral selector and an enhanced material, graphene–mesoporous silica–gold NP hybrids (GSGHs), as the electrochemical sensing platform. Herein, the introduction of GSGHs can enrich a number of aptamers, which can effectively enhance the detection limit of VP. The prepared GSGHs-based sensors have a wide linear range from 5 ng mL^{-1} to 56.5 μg mL^{-1}, with a low detection limit of 5 ng mL^{-1}.

Scheme 1A shows our typical sensing mechanism. The sequence of D-VP aptamer was split into two fragments named P1 and P2. The fragment P1 modified with thiol is the capture

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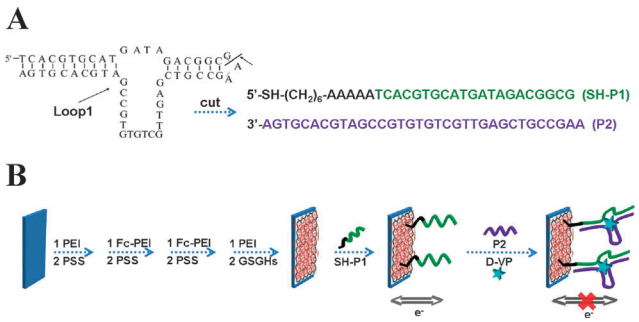

Scheme 1 (A) The secondary structure of the D-VP binding 55-mer DNA aptamer, the aptamer 21-mer and 34-mer DNA. The specific D-vasopressin enantiomer binding sites is the asymmetric internal loop of 20-mer nucleotides (Loop1). (B) Schematic representation of the sensing procedure for the analysis of D-VP based on the sensing platform with GSGHs as enhanced materials and Fc-PEI as electrochemical probe.

probe and immobilized on the sensing platform. The fragment P2 is the detection probe which can conjunct with the capture probe to fold into a three-way junction in the presence of D-VP. In order to effectively magnify the above sensing process, GSGHs were used as enhancing materials to capture more D-VP aptamers, as shown in Scheme 1B. In the magnified strategy, electrochemical probe ferrocene (Fc) was covalently appended on poly(ethyleneimine) (PEI) to form an Fc–PEI complex. Then, through layer-by-layer (LBL) assembly of Fc-PEI and poly(sodium 4-styrenesulfonate) (PSS) (0.5 mg mL^{-1}, containing 0.5 M NaCl), Fc molecules were immobilized on the negatively charged indium tin oxide (ITO) electrode to obtain a solid-state probe. The above assembly process has been successfully demonstrated in our recent paper.[9] We chose the bilayer number (n) to be 2 (which was enough for introducing electrochemical probe and meantime immobilizing GSGHs, having no influences on the conductivity between the probe and ITO electrode) and further used another layer of PEI as the outermost layer to fix the GSGHs. The multilayer was then dried for 30 min, after which 15 μL of 12.5 mg mL^{-1} GSGHs was cast on the multilayer and then used to enrich the capture probes. Scanning electron microscopy (SEM) was performed to characterize the morphology of the sensing platform, as shown in Fig. S1 (ESI†). A great number of nanosheets with rough surface were observed. Fig. S2 (ESI†) shows a typical transmission electron microscopy (TEM) image of GSGHs. A number of gold nanoparticles are supported on the surface of nanosheets, which could be used to enrich the captured probe and thus enhance the sensitivity. It should be noted that the obtained GSGHs have some obvious advantages over individual graphene or gold nanoparticles, and also two-component graphene/silica or graphene/Au hybrids.[9] (a) Mesoporous silica itself can reduce the nonspecific adsorption of the DNA. (b) GSGHs have good electronic conductivity, which facilitate the electronic transfer of probe molecule. (c) GSGHs can enlarge the electrode area, which is important for enhancing the detection performance. Cyclic voltammetry (CV) measurements were first employed to examine the formation of the sensing platform. As shown in Fig. S3 (ESI†), $(Fc\text{-}PEI/PSS)_1$ exhibited a small peak current whereas $(Fc\text{-}PEI/PSS)_2$ showed a larger peak current. After the GSGHs were immobilized onto $(Fc\text{-}PEI/PSS)_2$, the peak current of probe increased significantly, because GSGHs themselves could provide high surface area and excellent conductivity for enhancing electrochemical signals of probe molecules. The peak current of the PEI/PSS/$(Fc\text{-}PEI/PSS)_2$/PEI/GSGHs-coated electrode exhibited a linear dependence on the scan rate in the range of 10–130 mV s^{-1}, which confirmed the surface-confined species as the origin of the redox reaction (Fig. S4, ESI†). In addition, the stability of the sensing platform was investigated. The multi-layer of PEI/PSS/$(Fc\text{-}PEI/PSS)_2$/PEI/GSGHs was left dried and air-exposed at room temperature over 10 days, and the peak current only decreased by 6.6%, showing that the multilayer was stable.

After the fabrication of the GSGHs sensing platform, the thiolated P1 (SH-P1) was covalently labeled onto the gold NPs (attached on GSGHs) through Au–S bond. 6-Mercaptohexanol (MCH) was used as blocker to make the array of SH-P1 on the electrode interface more regular. Then, the mixture of target D-VP and P2 were covered on the sensing platform and the SH-P1 layer was partly hybridized with P2. The resulting complex was used to bind the D-VP, leading to a decreased electrochemical signal of Fc-PEI. CV was firstly used to validate the fabrication of the sensing interface and the detection of D-VP. As shown in Fig. 1A, the GSGHs sensing platform showed a large current of Fc. After assembling SH-P1, the CV response was reduced obviously. After MCH assembly, the CV response was further reduced. By dropping 50 μL samples containing 5 μM P2 and D-VP on the prepared electrode for further reaction, the CV response kept on decreasing, indicating the SH-P1 partly hybridized with P2 was linked onto the target. The corresponding

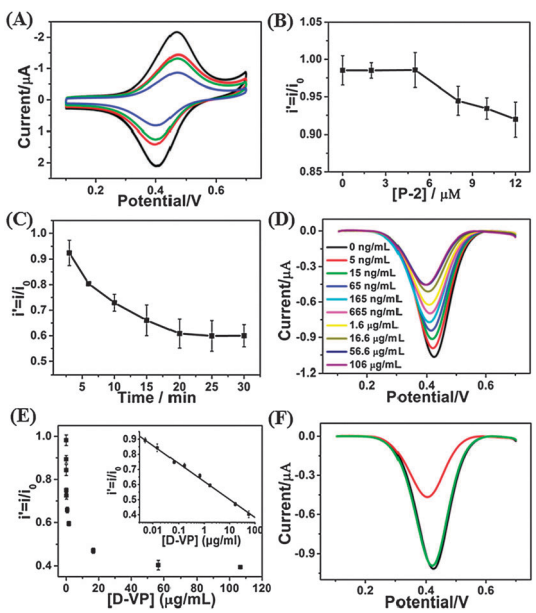

Fig. 1 (A) CVs of the GSGHs-based sensing platform before (blank) and after functionalization with SH-P1 (red), SH-P1 + MCH (green) and SH-P1 + MCH + 10 μg mL^{-1} D-VP (blue). (B) The response of biosensor with different concentrations of P2 when D-VP was absent. (C) The time-dependent DPV response for D-VP detection. (D) The DPV response of sensing interface for different concentration of D-VP. (E) The relative decreased response to different concentrations of D-VP. (F) The selectivity of the sensor for D-VP.

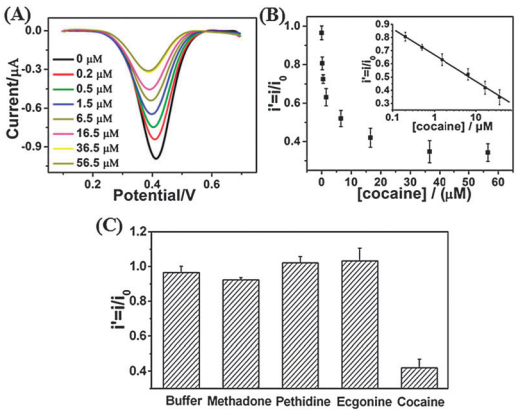

Fig. 2 (A) The relative DPV responses of the biosensor to different concentration of cocaine (from 0.2 to 56.5 μM). (B) The relative responses to cocaine with different concentrations (from 0.2 μM to 56.5 μM). Inset: a linear range from 0.2 to 36.5 μM. (C) Control experiment for buffer, 2.5 mM methadone, 2.5 mM pethidine, 2.5 mM ecgonine and 16.5 μM cocaine.

SH-P1/D-VP/P2 complex was attached on the sensing platform, resulting in blocking the electrode surface with a partially covered adsorption layer. The larger the coverage, the slower the apparent electron transfer will be, consistent with the partially blocked electrode theoretics.[10]

Before testing the D-VP, we first studied the effect of both the concentration of P2 and the operation time on peak current of the probe (Fig. 1B). The sensing interfaces (PEI/PSS/(Fc-PEI/PSS)$_2$/PEI/GSGHs/SH-P1/MCH) were treated with 50 μL of different concentrations of P2 solution (without D-VP) at room temperature. When the concentration of P2 was over 5 μM (Fig. 1B), an obvious peak current decrease (i/i_0, i represents the cathodic peak current detected after each target was introduced and i_0 represents the blank cathodic peak current of the sensing interface) was observed. As we know, the split aptamer would equilibrate between its two dissociated parts and a folded complex.[11] When the concentration of the two strands were high enough, they could hybridize by themselves without target. Thus, 5 μM P2 was employed throughout the detection process. In the kinetic experiment (Fig. 1C), the peak current decreased significantly with time when 2 μg mL^{-1} D-VP was present (with 5 μM P2), and reached a plateau at 20 min. Therefore, 20 min was chosen as the detection time.

In the quantitative analysis, the prepared sensing interface was treated with different amounts of D-VP in the presence of 5 μM P2. After the 20-min incubation, SH-P1/D-VP/P2 complex was formed on the sensing interface, which led to the decrease of DPV to different degrees. Fig. 1D showed that with increasing the concentration of D-VP, the DPV signals decreased obviously. A linear range from 5 ng mL^{-1} to 56.5 μg mL^{-1} (R^2 = 0.997) was obtained with a detection limit of 5 ng mL^{-1} (Fig. 1E). The present sensor also shows good selectivity (Fig. 1F). It was observed that 100 μg mL^{-1} L-VP (green line) showed almost no current change compared with the original one (blank line) while 20 μg mL^{-1} D-VP led to an evident DPV change (red line), indicating this strategy can distinguish enantiomers and detect one of the enantiomers (here, D-enantiomer) with high specificity and sensitivity.

To demonstrate the generality of the sensing strategy using GSGHs as magnified materials, we attempted to fabricate another split aptamer (with two fragments SH-C1 and C2) using cocaine as a target to testify the applicability of the GSGHs-based sensing platform. The detection principle was similar to that of aforementioned D-VP. As demonstrated in Fig. 2A and B, the aptamer-appended multilayer film could detect cocaine in a wide range of 0.2 to 36.5 μM with a detection limit of 0.2 μM. For the selectivity (Fig. 2C), two analgesic drugs (pethidine and methadone) and the complete hydrolyzate of cocaine (ecgonine) were used as controls. 16.5 μM cocaine led to an evident color change whereas 2.5 mM ecgonine, 2.5 mM pethidine, and 2.5 mM methadone did not cause observable DPV change, suggesting the good selectivity for cocaine of the present sensor.

In summary, by using a multifunctional sensing platform combining the magnified material (GSGHs) with Fc-PEI (Fc, redox probe), we firstly reported a designed split aptamer as a new chiral selector for distinguishing VP enantiomers *via* an electrochemical technique. The proposed sensing platform has high sensitivity (the detection limit is 5 ng mL^{-1} for D-VP) and excellent selectivity (could distinguish L-VP). Herein, GSGHs themselves could provide high surface area and excellent conductivity for enhancing electrochemical signals of probe molecules, thus leading to the enhanced sensitivity. Most importantly, the present sensing strategy is a general method for detection of different aptamer-based analytes (here, cocaine as a model).

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